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# The Use of Some Selected Antioxidant Supplements in the Management of Fluoride Toxicity on Adult Male Wistar Rats' Fecundity

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**Abstract:** The use of ameliorative antioxidant potentials of some selected antioxidant supplements in the management of fluoride toxicity on adult male Wistar rats' fecundity was investigated. Thirty-six adult male albino rats were divided into 6 groups. Group I (positive control) received oral dose of normal feed and water for 4 weeks and 6 weeks, Group II (negative control) sodium fluoride (10mg/kg) body weight (b.w), Group III received sodium fluoride (10mg/kg) + selenium(0.5mg/kg) b.w, Group IV sodium fluoride (10mg/kg) + zinc (14.8mg/kg) b.w, Group V sodium fluoride (10mg/kg) + ginseng (10mg/kg) b.w, Group VI sodium fluoride (10mg/kg) + vitamin E (100mg/kg) b.w. At the end of the 4th and 6th week, hormone assay: (testosterone, prolactin, luteinizing hormone and follicle stimulating hormone, sperm quality analysis: morphology, motility, abnormality, sluggishness, deadness and sperm count) were analysed. Result showed that Group 11 had a significant decrease ($p<0.05$) in the sperm quality parameters and a significant decrease ($p<0.05$) in the hormone parameters compared to the positive control group. However, there was no significant difference in the sperm sluggishness parameter after 4 weeks and 6 weeks and also no significant difference in the sperm abnormality parameter after 4 weeks. Testicular examination of group 11(Sodium fluoride only) showed necrosis, epithelial apoptosis, and atrophied seminiferous tubules. Concomitant administration of sodium fluoride +selenium, sodium fluoride + zinc, sodium fluoride + ginseng, and sodium fluoride + vitamin E respectively showed a significant reversal of the toxic effect of NaF on the treated animals and this corroborated with the histology results of the testes. The result showed that sodium fluoride induced severe toxic changes in the male reproductive system while these supplements provided partial and complete amelioration against these toxic effects.

**Keywords:** Fluoride toxicity; Antioxidants; Amelioration; Fecundity.

## 1. Introduction

The human population is exposed to sodium fluoride (NaF) from a number of sources, including water, medicines, pesticides, insecticides, fertilizer residues, dental restorative materials, dental products (tooth pastes and mouth rinses), paediatric supplements, beverages prepared with fluoridated water and food [1]. Fluorosis is an endemic public health problem in nearly 22 nations around the world. The World Health Organization (WHO) guideline is that 1.5 ppm of fluoride is the desirable upper limit in drinking water. Fluoride is an essential element needed for normal development and growth of animals and human beings. Exposure to fluoride is widespread and its main source in humans is drinking water, in which it is either present naturally in groundwater or due to community water fluoridation. In addition, fluoridated salt and fluoride-containing food, such as fish, may contribute to high-dietary fluoride intake. Dietary supplements are also available for use by persons living in non-fluoridated areas to increase their fluoride exposure. Another common source of fluoride is the use of toothpaste, mouthwash solutions, fluoride gels, and other topical sources [2, 3] . Moreover, the atmosphere may contain airborne fluoride arising from soils, industry, coal fires, and volcanoes. It was estimated that the average intake of fluoride through food consumption is approximately 2 mg/day for adults. By the use of fluoridated water and salt, the fluoride intake could reach 6 mg/day, without taking into account toothpaste use [4]. However, reports by Huang, *et al.* [5] on the effects of fluoride on reproductive functions were not fully understood, and reported findings were conflicting.

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Selenium is one of the essential trace elements for both human and animals. Scientists documented the vital role of selenium in numerous biological functions mainly through its antioxidant effects. They also claim that it has an immunomodulatory, anticarcinogenic and antiatherogenic activities [6, 7]. Vitamin E (vit E) is believed to exert its protective effect at the cellular-molecular level, primarily through destruction of cell damaging free radical oxygen species [8]. Zinc antagonizes oxidative stress, apoptosis, and cell cycle changes induced by excess fluoride [9]. Ginseng (the root of Panax ginseng) is one of the most commonly used herbal medicines in Asian and Western countries. Studies have shown a wide range of beneficial effects of ginseng against human diseases [10, 11].

Considering that fluorosis is a public health issue and the very fact that fluoride exposure has a definite effect on reproduction. The following study was planned to observe the toxic effect induced by sodium fluoride on the reproductive hormone profile, sperm quality and histology of testis and to evaluate the ameliorative role, if any of selenium, zinc, ginseng and vitamin E against these toxic effects in the reproductive health of adult male albino rats.

# 2. Materials and Methods

## 2.1 Drugs and Reagents

Sodium fluoride was obtained in the form of white powder from JoeChem Ventures, Rumuchakara, Choba Road, Port- Harcourt, Nigeria. Selenium, Zinc, Ginseng and Vitamin E in the form of supplement drugs, were obtained from Chux Medical, 1 Alogu Road Alakahia Port- Harcourt, Nigeria. These drugs were freshly prepared by dissolving each of them in distilled water and given by oral gavage. Hormonal kits for determination of rat hormonal concentrations in plasma are products of Boditech Med Incorporated, 43 Geodudanjin1-gil, Dongnae-myeon, Chuncheon-si, Gang-won-do 200-883, Republic of Korea and were obtained from HDL Medical and Laboratory Support Group, 4 Chief Ejims Street, Rumuomasi, PortHarcourt, Nigeria. All other reagents were of analytical grade.

## 2.2. Animals

Thirty-six sexually matured male wistar albino rats weighing 150-200gm were obtained from the Animal House of College of Natural and Applied Sciences, Department of Animal and Environmental Biology, University of Port Harcourt. The animals were divided into six equal groups.

## 2.3. Experimental Design

The animals were divided into six groups of six rats each. Group I (positive control) received oral dose of normal feed and water for 4 weeks and 6 weeks, Group II (negative control) sodium fluoride (10mg/kg) b.w, Group III received sodium fluoride (10mg/kg) + selenium(0.5mg/kg) b.w, Group IV sodium fluoride (10mg/kg) + zinc (14.8mg/kg) b.w, Group V sodium fluoride (10mg/kg) + ginseng (10mg/kg) b.w, Group VI sodium fluoride (10mg/kg) + vitamin E (100mg/kg) b.w.

## 2.4. Fluoride/ Drug administration

The rats were sorted into six groups of six animals each on weight basis. The animals were acclimatized for a period of 2 weeks on guinea growers mash (Port Harcourt Flour Mills, Port Harcourt, Nigeria), static bioassay tests were conducted to find out the value of lethal dose, LD 50. The present study reveals the lethal dose for the 50% of the populations for a month was 10mg/kg so it was undertaken for study. Treatment commenced using the experimental design.

## 2.5. Collection of Blood and Tissue Samples

The procedure used was described by Yakubu, *et al.* [12]. At the end of the $4^{th}$ and $6^{th}$ week, each of the adult rats was anaesthetized in chloroform vapor in desiccators and dissected using surgical forceps and scissors. Blood samples were collected by cardiac puncture using sterile syringe and needle into plain sample tubes and were allowed to stand for 120mins at room temperature to clot, after which they were centrifuged at 3000rpm for 10mins using a bench top centrifuge Uniscope Laboratory Centrifuge (Model 802, Surgifriend Medicals and Essex, England), to obtain the serum. The sera obtained from the respective samples were carefully removed using Pasteur pipettes, into respective labeled plastic specimen bottles and stored frozen in a bio-freezer until ready for assessment of levels of Testosterone, Prolactin, FSH and LH. The semen was collected for semen analysis and the testes were dissected and submitted for histopathological examination.

## 2.6. Sperm Parameters

From each separated epididymis, the caudal part was removed and placed in a beaker containing 10 ml diluting solution (sodium bicarbonate; 5g and formalin neutral; 1 ml in 100 ml of distilled water). Each section was quickly macerated with a pair of sharp scissors and left for a few minutes to liberate its spermatozoa into the solution. Sperm count was done under the microscope using a new improved Neubauer Heamocytometer and the sperm count was calculated per epididymis. Semen drop was placed on the slide and two drops of warm 2.9% sodium citrate was added. The slide was covered with a cover slip and examined under the microscope using X40 objectives for sperm motility, morphology, abnormality, sluggishness and deadness [13].

## 2.7. Hormonal Assay

Plasma Testosterone, Prolactin, Follicle-stimulating and Luteinizing hormones were determined by fluorescence immunoassay (FIA) methods with commercial kits (Boditech Med Incorporated, Republic of Korea), using the ichroma machine (Boditech: BOD13303, Korea).

## 2.8. Histopathological Examination

Testes were removed after being freed from surrounding tissue. The tissues were fixed in 10% formal saline in labeled plain bottles for histological studies. The tissues were subjected to standard routine histological procedures as described by Brown [14]. The slides were viewed using the light microscope and histopathological changes were observed and recorded at X40 magnification identifying both the normal and atrophied seminiferous tubules and spermatocytes.

## 2.9. Statistical Analysis of Data

The Data for toxicological screening were analyzed for statistical differences between treatment groups, by means of One-Way ANOVA and post hoc LSD, on SPSS 19. In all p value of less than 0.05 ($p<0.05$) was considered to be significant. Data are presented as mean ± SD (standard deviation).

# 3. Result

## 3.1. Sperm Quality Parameters

**Table-1.** Mean ± SD of Sperm Morphology of the control and treatment groups after 4 and 6 weeks

| Morphology (%) | 4 weeks | 6 weeks |
|---|---|---|
| Control | $80.66 \pm 1.15^{a}$ | $85.00 \pm 5.00^{a}$ |
| NaF | $36.66 \pm 5.77^{a,b}$ | $36.66 \pm 2.88^{a,b}$ |
| NaF+Se | $65.00 \pm 5.00^{b}$ | $86.66 \pm 5.77^{b}$ |
| NaF+Zn | $53.33 \pm 7.63^{b}$ | $45.00 \pm 5.00$ |
| NaF+Ge | $80.00 \pm 5.00^{b}$ | $75.00 \pm 5.00^{b}$ |
| NaF+Vit E | $90.00 \pm 5.00^{b}$ | $90.00 \pm 5.00^{b}$ |

NaF= Sodium Fluoride, Se=Selenium, Zn=Zinc, Ge= Ginseng, Vit E= Vitamin E. n=3, per group/week. Values in the same column with common superscript letters (a,b,....) are significantly different at $p<0.05$. Superscript $A^{(a)}$ represents significant difference when group I (control group) are compared with group II (NaF group) at $p<0.05$. Superscript $B^{(b)}$ represents significant difference when group II (NaF group) are compared with the antioxidant treated groups at $p<0.05$. Values without superscripts indicate no significant difference when compared with the control and antioxidant groups.

**Table-2.** Mean ± S.D of Sperm Abnormality of the control and treatment groups after 4 and 6 weeks

| Abnormality (%) | 4 weeks | 6 weeks |
|---|---|---|
| Control | 20.00 ± 0.00 | $8.33 \pm 2.88^{a}$ |
| NaF | 35.00 ± 5.00 | $38.33 \pm 7.63^{a,b}$ |
| NaF+Se | 36.66± 5.77 | $11.66 \pm 2.88^{b}$ |
| NaF+Zn | 35.00± 22.91 | $56.66 \pm 5.77^{b}$ |
| NaF+Ge | 18.33± 2.88 | $21.66 \pm 2.88^{b}$ |
| NaF+Vit E | 8.33± 2.88 | $10.00 \pm 5.00^{b}$ |

NaF= Sodium Fluoride, Se=Selenium, Zn=Zinc, Ge= Ginseng, Vit E= Vitamin E. n=3, per group/week. Values in the same column with common superscript letters (a,b,....) are significantly different at $p<0.05$. Superscript $A^{(a)}$ represents significant difference when group I (control group) are compared with group II (NaF group) at $p<0.05$. Superscript $B^{(b)}$ represents significant difference when group II (NaF group) are compared with the antioxidant treated groups at $p<0.05$. Values without superscripts indicate no significant difference when compared with the control and antioxidant groups.

**Table-3.** Mean ± S.D of Sperm Motility of the control and treatment groups after 4 and 6 weeks

| Motility (%) | 4 weeks | 6 weeks |
|---|---|---|
| Control | $80.00 \pm 5.00^{a}$ | $78.33 \pm 2.88^{a}$ |
| NaF | $40.00 \pm 5.00^{a,b}$ | $38.33 \pm 2.88^{a,b}$ |
| NaF+Se | $48.33 \pm 2.88$ | $76.66 \pm 2.88^{b}$ |
| NaF+Zn | $40.00 \pm 10.00$ | $38.33 \pm 2.88$ |
| NaF+Ge | $75.00 \pm 5.00^{b}$ | $30.00 \pm 5.00$ |
| NaF+Vit E | $81.66 \pm 2.88^{b}$ | $80.00 \pm 5.00^{b}$ |

NaF= Sodium Fluoride, Se=Selenium, Zn=Zinc, Ge= Ginseng, Vit E= Vitamin E. n=3, per group/week. Values in the same column with common superscript letters (a,b,....) are significantly different at p<0.05. Superscript $A^{(a)}$ represents significant difference when group I (control group) are compared with group II (NaF group) at p<0.05. Superscript $B^{(b)}$ represents significant difference when group II (NaF group) are compared with the antioxidant treated groups at p<0.05. Values without superscripts indicate no significant difference when compared with the control and antioxidant groups.

**Table-4.** Mean ± S.D of the Sperm Deadness of the control and treatment groups after 4 and 6 weeks

| Deadness (%) | 4 weeks | 6 weeks |
|---|---|---|
| Control | $18.33 \pm 7.63^{a}$ | $13.33 \pm 2.88^{a}$ |
| NaF | $55.00 \pm 5.00^{a,b}$ | $53.33 \pm 2.88^{a,b}$ |
| NaF+Se | $41.66 \pm 2.88$ | $8.33 \pm 2.88^{b}$ |
| NaF+Zn | $33.33 \pm 10.40^{b}$ | $41.66 \pm 2.88^{b}$ |
| NaF+Ge | $11.66 \pm 2.88^{b}$ | $21.66 \pm 2.88^{b}$ |
| NaF+Vit E | $8.33 \pm 2.88^{b}$ | $11.66 \pm 2.88^{b}$ |

NaF= Sodium Fluoride, Se=Selenium, Zn=Zinc, Ge= Ginseng, Vit E= Vitamin E. n=3, per group/week. Values in the same column with common superscript letters (a,b,....) are significantly different at p<0.05. Superscript $A^{(a)}$ represents significant difference when group I (control group) are compared with group II (NaF group) at p<0.05. Superscript $B^{(b)}$ represents significant difference when group II (NaF group) are compared with the antioxidant treated groups at p<0.05. Values without superscripts indicate no significant difference when compared with the control and antioxidant groups.

**Table-5.** Mean ± S.D of the Sperm Sluggishness of the control and treatment groups after 4 and 6 weeks

| Sluggishness (%) | 4 weeks | 6 weeks |
|---|---|---|
| Control | $5.00 \pm 0.00$ | $8.33 \pm 2.88$ |
| NaF | $8.33 \pm 7.63$ | $13.33 \pm 2.88$ |
| NaF+Se | $10.00 \pm 5.00$ | $11.66 \pm 2.88$ |
| NaF+Zn | $16.66 \pm 7.63$ | $18.33 \pm 2.88$ |
| NaF+Ge | $13.33 \pm 2.88$ | $13.33 \pm 2.88$ |
| NaF+Vit E | $10.00 \pm 5.00$ | $6.66 \pm 2.88$ |

NaF= Sodium Fluoride, Se=Selenium, Zn=Zinc, Ge= Ginseng, Vit E= Vitamin E. n=3, per group/week. Values in the same column with common superscript letters (a,b,....) are significantly different at p<0.05. Superscript $A^{(a)}$ represents significant difference when group I (control group) are compared with group II (NaF group) at p<0.05. Superscript $B^{(b)}$ represents significant difference when group II (NaF group) are compared with the antioxidant treated groups at p<0.05. Values without superscripts indicate no significant difference when compared with the control and antioxidant groups.

**Table-6.** Mean ± S.D of the Sperm count of the control and treatment groups after 4 and 6 weeks

| Sperm count ($x10^{6}$/ml) | 4 weeks | 6 weeks |
|---|---|---|
| Control | $850.00 \pm 50.00^{a}$ | $750.00 \pm 50.00^{a}$ |
| NaF | $216.66 \pm 28.86^{a,b}$ | $233.33 \pm 28.86^{a,b}$ |
| NaF+Se | $350.00 \pm 50.00$ | $866.66 \pm 57.73^{b}$ |
| NaF+Zn | $316.66 \pm 104.08$ | $250.00 \pm 50.00$ |
| NaF+Ge | $750.00 \pm 50.00^{b}$ | $616.66 \pm 28.86^{b}$ |
| NaF+Vit E | $850.00 \pm 86.60^{b}$ | $916.66 \pm 28.86^{b}$ |

NaF= Sodium Fluoride, Se=Selenium, Zn=Zinc, Ge= Ginseng, Vit E= Vitamin E. n=3, per group/week. Values in the same column with common superscript letters (a,b,....) are significantly different at p<0.05. Superscript $A^{(a)}$ represents significant difference when group I (control group) are compared with group II (NaF group) at p<0.05. Superscript $B^{(b)}$ represents significant difference when group II (NaF group) are compared with the antioxidant treated groups at p<0.05. Values without superscripts indicate no significant difference when compared with the control and antioxidant groups.

## 3.2. Hormonal Parameters

**Table-7.** Mean ± SD of Testosterone concentration of control and test groups after 4 weeks and 6 weeks.

| Testosterone (ng/ml) | 4 weeks | 6 weeks |
|---|---|---|
| Control | 2.73 ± 0.11[a] | 2.76 ± 0.25 |
| NaF | 1.60 ± 0.17[a,b] | 1.86 ± 0.05[b] |
| NaF+Se | 4.73 ± 0.25[b] | 6.90 ± 3.40[b] |
| NaF+Zn | 1.76± 0.05[b] | 6.53 ± 1.32[b] |
| NaF+Ge | 2.56 ± 0.15[b] | 2.70 ± 0.75 |
| NaF+Vit E | 5.03 ± 0.15[b] | 6.76 ± 0.66[b] |

NaF= Sodium Fluoride, Se=Selenium, Zn=Zinc, Ge= Ginseng, Vit E= Vitamin E. n=3, per group/week. Values in the same column with common superscript letters (a,b,....) are significantly different at p<0.05. Superscript A[(a)] represents significant difference when group I (control group) are compared with group II (NaF group) at p<0.05. Superscript B[(b)] represents significant difference when group II (NaF group) are compared with the antioxidant treated groups at p<0.05. Values without superscripts indicate no significant difference when compared with the control and antioxidant groups.

**Table-8.** Mean ± SD of Prolactin concentration of control and test groups after 4 weeks and 6 weeks.

| Prolactin (ng/ml) | 4 weeks | 6 weeks |
|---|---|---|
| Control | 2.30 ± 0.36[a] | 2.69 ± 0.07[a] |
| NaF | 0.65 ± 0.06[a,b] | 0.42 ± 0.01[a,b] |
| NaF+Se | 1.39 ± 0.01[b] | 1.64 ± 0.10[b] |
| NaF+Zn | 0.70± 0.00[b] | 0.82 ± 0.04[b] |
| NaF+Ge | 1.40 ± 0.18[b] | 1.38 ± 0.02[b] |
| NaF+Vit E | 2.39 ± 0.18[b] | 2.64 ± 0.16[b] |

NaF= Sodium Fluoride, Se=Selenium, Zn=Zinc, Ge= Ginseng, Vit E= Vitamin E. n=3, per group/week. Values in the same column with common superscript letters (a,b,....) are significantly different at p<0.05. Superscript A[(a)] represents significant difference when group I (control group) are compared with group II (NaF group) at p<0.05. Superscript B[(b)] represents significant difference when group II (NaF group) are compared with the antioxidant treated groups at p<0.05. Values without superscripts indicate no significant difference when compared with the control and antioxidant groups.

**Table-9.** Shows the Mean ± SD of Luteinizing hormone concentration of control and test groups after 4 weeks and 6 weeks.

| Luteinizing hormone (ng/ml) | 4 weeks | 6 weeks |
|---|---|---|
| Control | 0.87 ± 0.07[a] | 1.80 ± 0.03[a] |
| NaF | 0.27 ± 0.06[a,b] | 0.34 ± 0.08[a,b] |
| NaF+Se | 0.61 ± 0.09[b] | 0.63 ± 0.08[b] |
| NaF+Zn | 0.33 ± 0.03 | 0.42 ± 0.04 |
| NaF+Ge | 0.62 ± 0.02[b] | 0.96 ± 0.12[b] |
| NaF+Vit E | 1.01 ± 0.16[b] | 1.12 ± 0.10[b] |

NaF= Sodium Fluoride, Se=Selenium, Zn=Zinc, Ge= Ginseng, Vit E= Vitamin E. n=3, per group/week. Values in the same column with common superscript letters (a,b,....) are significantly different at p<0.05. Superscript A[(a)] represents significant difference when group I (control group) are compared with group II (NaF group) at p<0.05. Superscript B[(b)] represents significant difference when group II (NaF group) are compared with the antioxidant treated groups at p<0.05. Values without superscripts indicate no significant difference when compared with the control and antioxidant groups.

**Table-10.** Shows the Mean ± SD of Follicle-stimulating hormone concentration of control and test groups after 4 weeks and 6 weeks.

| Follicle-stimulating hormone (ng/ml) | 4 weeks | 6 weeks |
|---|---|---|
| Control | 0.76 ± 0.05[a] | 2.53 ± 0.05[a] |
| NaF | 0.30 ± 0.02[a,b] | 1.01 ± 0.24[a,b] |
| NaF+Se | 0.77 ± 0.02[b] | 1.83 ± 0.06[b] |
| NaF+Zn | 0.38 ± 0.05 | 0.93 ± 0.10 |
| NaF+Ge | 0.79 ± 0.01[b] | 1.79 ± 0.01[b] |
| NaF+Vit E | 0.88 ± 0.02[b] | 2.16 ± 0.18[b] |

NaF= Sodium Fluoride, Se=Selenium, Zn=Zinc, Ge= Ginseng, Vit E= Vitamin E. n=3, per group/week. Values in the same column with common superscript letters (a,b,....) are significantly different at $p<0.05$. Superscript $A^{(a)}$ represents significant difference when group I (control group) are compared with group II (NaF group) at $p<0.05$. Superscript $B^{(b)}$ represents significant difference when group II (NaF group) are compared with the antioxidant treated groups at $p<0.05$. Values without superscripts indicate no significant difference when compared with the control and antioxidant groups.

## 3.3. Histopathology of Testis

**Photomicrograph of the architecture of the testis of the control as well as the groups ameliorated with various antioxidants (Plate 1) was taken after 4 weeks.**

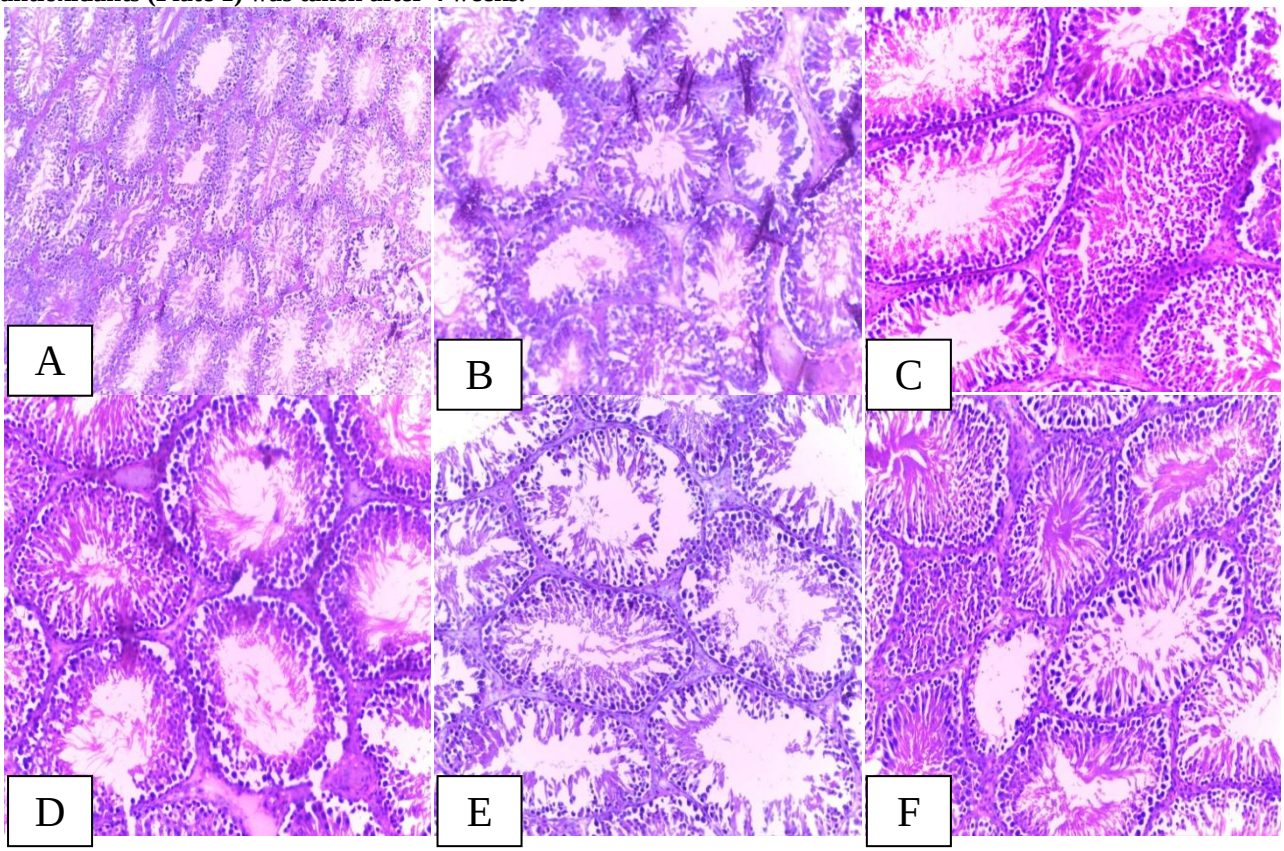


**Plate-1.** Histopathological changes of the architecture of the testis of the control as well as the groups ameliorated with various antioxidants after 4 weeks.

**A (Control group):** showed normal spermatogenesis with different stages of differentiation and maturation. Spermatozoa were found in groups attached to the inner aspect of the lumen of the seminiferous tubules. **B** (**NaF-treated group):** showed lack of differentiation and maturation of spermatocytes and marked infiltration in the interstitial area of seminiferous tubules. Very minute quantities of spermatozoa were seen in the lumen of the seminiferous tubules; reduction in sperm cells and tubular damage. **C** (**NaF+Se-treated group):** showed increase in sperm cells in some tubules as compared to the NaF-treated group. **D** (**NaF+Zn-treated group):** showed mild tubular changes and little increase in the number of sperm cells. **E** (**NaF+Ge-treated group):** showed increase in number of maturing sperm cells. There was normal spermatogenesis in some of the seminiferous tubules. **F** (**NaF+Vit E-treated group):** showed marked increase in the number of sperm cells.

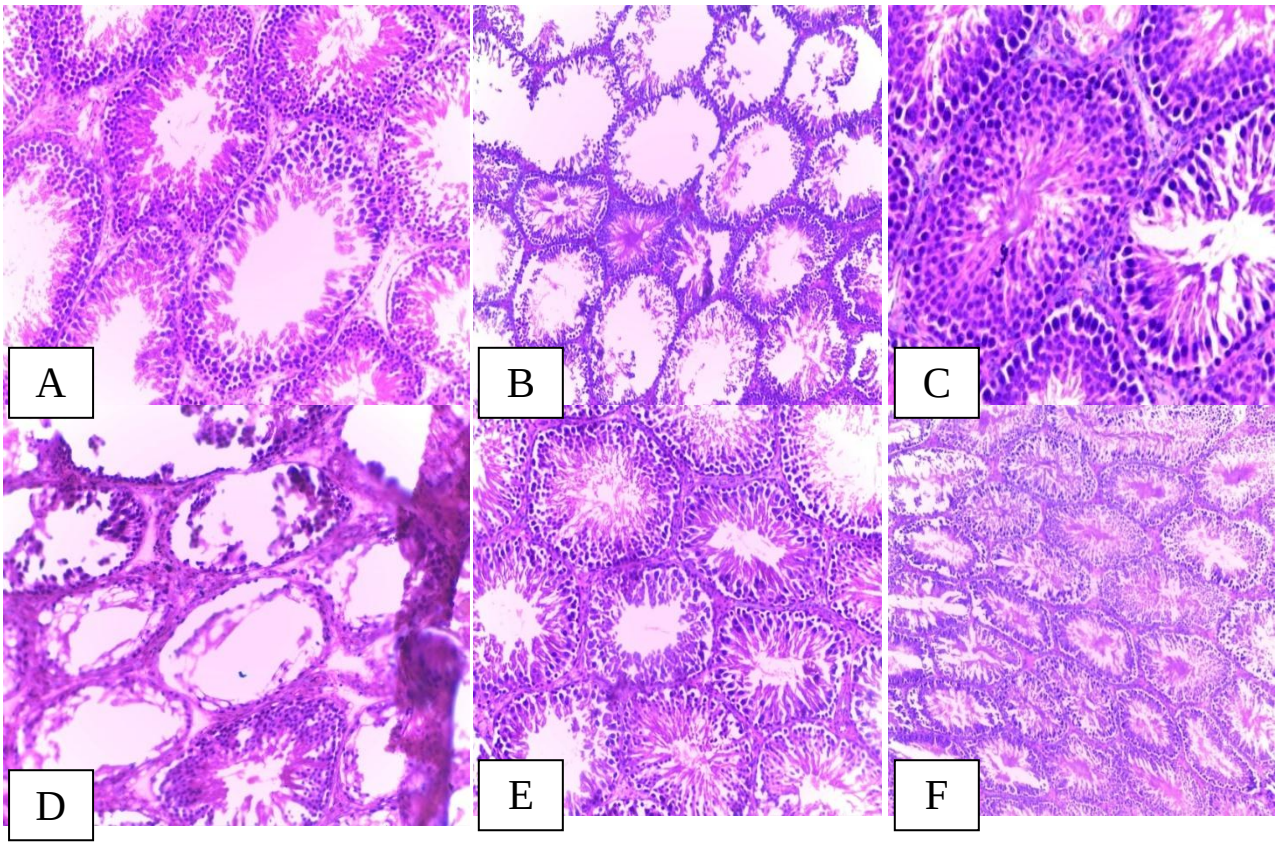


**Plate-2.** Histopathological changes of the architecture of the testis of the control as well as the groups ameliorated with various antioxidants after 6 weeks.

**A (Control group):** showed normal testicular microarchitecture with spermatozoa present in groups attached to the inner aspect of the lumen of the seminiferous tubules. B (NaF-treated group): Showed tubular damage and reduction in sperm cells, lack of differentiation and maturation of spermatocytes with significant infiltration in the interstitial area of the seminiferous tubules. C (NaF+Se-treated group): showed increase in the number of sperm cells with normal spermatogenesis and with different stages of differentiation and maturation. D (NaF+Zn-treated group): showed tubular damage and marked reduction in number of sperm cells. E (NaF+Ge-treated group): showed increase in the number of sperm cells. Some sperm cells were seen in groups attached to the inner aspect of the lumen of the seminiferous tubules. F (NaF+Vit E-treated group): showed remarkable increase in the number of sperm cells; spermatogenesis occurred with different stages of differentiation and maturation.

## 4. Discussion and Conclusion

Recent reports concerning fluoride adverse effects on the male reproductive system have attracted considerable interest. Clinical investigations and animal experiments suggested that fluoride can cause impairment of reproductive function [15, 16]. Selenium, Ginseng, Zinc and Vitamin E are essential elements in nutrition for the prevention of diseases in humans. Previous studies showed the essentiality of selenium in the body's antioxidant defence; thyroid hormone function; immune system function; formation of sperms; and functioning of the prostate gland [17] Vitamin E (vit E) is believed to exert its protective effect at the cellular-molecular level, primarily through destruction of cell damaging free radical oxygen species [8]. Zinc antagonizes oxidative stress, apoptosis, and cell cycle changes induced by excess fluoride [9]. Studies have shown a wide range of beneficial effects of ginseng against human diseases [10, 11]. The potential therapeutic effects of ginseng have been attributed to its immunostimulatory, antioxidant, and anti-inflammatory activities. [18, 19]. On exposure to 10 mg/kg body weight sodium fluoride for 4 weeks, there was a significant ($p<0.05$) decrease in the epididymal sperm count of the NaF-treated group (216.66 ± 28.86) as compared to the control group (850.00 ± 50.00) in this study but there was a significant increase in the sperm count when sodium fluoride-treated group was combined with vitamin E (850.00 ± 86.60) and with ginseng (750.00 ± 50.00) but no significant difference ($p>0.05$) when combined with zinc (316.66 ± 104.08) and with selenium (350.00 ± 50.00) compared to NaF-treated group (216.66 ± 28.86). After 6 weeks , there was a significant ($p<0.05$) decrease in the epididymal sperm count of the NaF-treated groups (233.33 ± 28.86) as compared to the control group (750.00 ± 50.00) in this study but there was a significant increase in the NaF+VitE-treated group (916.66±28.86), NaF+Se-treated group (866.66±5.73) and NaF+Ge-treated group (616.66±28.86) but no significant difference ($p<0.05$) with NaF+Zn-treated group (250.00 ± 50.00) compared to the NaF-treated group (233.33 ± 28.86). Most significant improvement was seen in the NaF+Vit E-treated group when compared to other treated groups. The observations in this study were similar with studies done by other workers who reported that there was a

decline in sperm count as compared to control in rats exposed to sodium fluoride 10 mg/kg body weight for 50 days [20], or with 10 mg/kg fluoride for 30 days in male mice [21]. Effect of fluoride toxicity on spermatogenesis could be because fluoride reduces testosterone levels by diminishing positive signals for its production and by reducing testicular zinc levels that impairs the angiotensin converting enzyme (ACE) activity and hence causes inhibition of spermatogenesis [1]. Apart from direct effect, fluoride inhibits androgen receptor (AR) mRNA expression in Sertoli cells and cause decrease in AR through which testosterone acts [5].

According to Ghosh, *et al.* [22] vitamin E, at the cellular level, is believed to exert its protective effect primarily through destruction of cell damaging free radical oxygen species produced by fluoride. Hence, vitamin E that is well-known antioxidant ameliorates fluoride induced toxicity due to its antioxidant and detoxification properties. Similarly, Chinoy and Sharma [21] reported a significant improvement in the mice sperm count in a group fed on combined vitamin D and E along with fluoride for 30 days as compared to group fed on fluoride 10 mg/kg for 30 days.

In this study, there was a significant decrease ($p<0.05$) in sperm motility (40.00 ± 5.00) in the group fed with fluoride10mg/kg for 4 weeks as compared to control (80.00 ± 5.00) but there was a significant increase in the sperm motility of the NaF+Vit E-treated group (81.66 ± 2.88) and NaF+Ge-treated group (75.00 ± 5.00) but no significant difference in the NaF+Se-treated group (48.33 ± 2.88) and NaF+Zn-treated group(40.00 ± 10.00) compared to the NaF-treated group (40.00 ± 5.00). After 6 weeks, there was a significant decrease ($p<0.05$) in the sperm motility of the NaF-treated group (38.33 ± 2.88) compared to the control group (78.33 ± 2.88) but there was a significant increase in the NaF+Vit E-treated group (80.00 ± 5.00) and NaF+Se-treated group (76.66 ± 2.88) but no significant difference in the NaF+Ge-treated group (30.00 ± 5.00) and NaF+Zn-treated group (38.33± 2.88) when compared to the NaF-treated group (38.33 ± 2.88). Vit E was most effective in ameliorating the NaF induced effect compared to other treated groups. There was a significant decrease ($p<0.05$) in morphology (36.66 ± 5.77) in group fed with fluoride10 mg/kg for 4 weeks as compared to control (80.66 ± 1.15) but there was a significant increase in the NaF+Vit E-treated group (90.00 ± 5.00), NaF+Se-treated group (65.00 ± 5.00), NaF+Ge-treated group (80.00 ± 5.00) and NaF+Zn-treated group (53.33 ± 7.63) when compared to the NaF-treated group (36.66 ± 5.77). Morphology of the NaF-treated group (36.66 ± 2.88) after 6 weeks, also revealed a significant decrease ($p<0.05$) when compared to the control group (85.00 ± 5.00) but there was a significant increase in the NaF+Vit E-treated group (90.00 ± 5.00), NaF+Se-treated group (86.66 ± 5.77) and NaF+Ge-treated group (75.00 ± 5.00) but no significant difference in the NaF+Zn-treated group compared to the NaF-treated group (36.66 ± 2.88). Vit E was most effective in ameliorating the NaF induced effect compared to other treated groups.

After 4 weeks, there was no significant difference ($p>0.05$) in the sperm abnormality of the NaF-treated group (35.00± 5.00) when compared to the control group (20.00 ± 0.00) and also no significant difference in the NaF+Vit E-treated group (8.33 ± 2.88), NaF+Se-treated group (36.66 ± 5.77), NaF+Ge-treated group (18.33 ± 2.88) and NaF+Zn-treated group (35.00 ± 22.91) compared to the NaF-treated group (35.00 ± 5.00). After 6 weeks, there was a significant increase ($p<0.05$) in the sperm abnormality of the NaF-treated group (38.33±7.63) when compared to the control group (8.33 ± 2.88) but there was a significant decrease in the NaF+Vit E-treated group (10.00 ± 5.00), NaF+Se-treated group (11.66 ± 2.88), NaF+Ge-treated group (21.66 ± 2.88) but a significant increase in the NaF+Zn-treated group (56.66± 5.77) compared to the NaF-treated group (38.33 ± 7.63). Vit E was most effective in ameliorating the NaF induced effect compared to other treated groups. After 4 weeks, there was a significant increase ($p<0.05$) in the sperm deadness of the NaF-treated group (55.00 ± 5.00) compared to the control group (18.33 ± 7.63) but a significant decrease in the NaF+Vit E-treated group (8.33 ± 2.88), NaF+Ge-treated group (11.66 ± 2.88) and NaF+Zn-treated group (33.33 ± 10.40) but no significant difference in the NaF+Se-treated group (41.66 ± 2.88) compared to the NaF-treated group (55.00 ± 5.00). After 6 weeks, there was also a significant increase ($p<0.05$) in the sperm deadness of the NaF-treated group (53.33 ± 2.88) compared to the control group (13.33 ± 2.88) but there was a significant decrease in the NaF+Vit E-treated group (11.66 ± 2.88), NaF+Se-treated group (8.33 ± 2.88), NaF+Ge-treated group(21.66 ± 2.88) and NaF+Zn-treated group (41.66 ± 2.88) compared to the NaF-treated group (53.33 ± 2.88). Most significant improvement was seen in the NaF+Vit E-treated group and NaF+Se-treated group in the $4^{th}$ and $6^{th}$ weeks respectively. After 4 weeks, there was no significant difference ($p>0.05$) in the sperm sluggishness of the NaF-treated group (8.33 ± 7.63) when compared to the control group (5.00 ± 0.00) as well as in the NaF+Vit E-treated group (10.00 ± 5.00), NaF+Se-treated group (10.00 ± 5.00), NaF+Ge-treated group (13.33 ± 2.88) and NaF+Zn-treated group(16.66 ± 7.63) when compared to the NaF-treated group (8.33 ± 7.63). After 6 weeks, there was also no significant difference ($p>0.05$) in the sperm sluggishness of the NaF-treated group (13.33 ± 2.88) when compared to the control group (8.33 ± 2.88) and no significant difference in the NaF+Vit E-treated group (6.66 ± 2.88), NaF+Se-treated group (11.66 ± 2.88), NaF+Ge-treated group (13.33 ± 2.88) and NaF+Zn-treated group (18.33 ± 2.88) compared to the NaF-treated group (13.33 ± 2.88). These observations are consistent with the work of Zhang, *et al.* [23] which stated that sperm motility and the production and emission of testosterone in male rats were adversely affected by exposure to fluoride. The changes in the testis tissue and in serum testosterone may be one of the pathways that lead to low sperm motility of male rats. Huang, *et al.* [5], reported a significant reduction in sperm motility of mice fed on 100, 200, and 300 mg NaF/L for 8 weeks as compared to the control group. Similarly, a study by Chinoy and Sharma [21] showed a significant ($p<0.001$) decrease on exposure of 10 mg/kg body weight of fluoride for 30 days in mice. Effect of fluoride (30 mM) on the metabolism and motility of ejaculated bull sperm *in vitro* had also been demonstrated, the sperms became immotile

within 2 min at 30°C. Similarly, human spermatozoa lost their motility *in vitro* in the presence of 250 mM Fluoride within 20 min of exposure [20].

According to Chinoy, *et al.* [20] and Zakrzewska, *et al.* [24] the mechanism by which fluoride affects sperm motility has not been clearly elucidated. However, it has been postulated that fluoride could act directly on motile apparatus without affecting other metabolic systems. Also fluoride binds with cofactors like Mg, Ca, Zn, and Se and inhibits glycolysis, respiration and motility of sperms. Thus, there could be decline in fructose level due to alteration in carbohydrate metabolism after fluoride treatment. Another reason for decreased sperm motility was decreased level of androgen carrier proteins involved in sperm motility Chinoy, *et al.* [25]. Chinoy and Sharma reported that in group fed on vitamin D during the withdrawal period of 30 days after exposure to 10 mg/kg body weight NaF for the same period, a statistically significant improvement in sperm motility was seen compared to control. At the cellular level, Vit E is believed to exert its protective effect primarily through destruction of cell damaging free radical oxygen species produced by fluoride. Hence, vitamin E that is well-known antioxidant ameliorates fluoride-induced toxicity due to its antioxidant and detoxification properties. Narayana and Chinoy [26] studied the effects of ingestion of sodium fluoride (NaF), 10 mg/kg body weight for 50 days, on the structure and metabolism of sperm of albino rats (Rattus norvegicus). In different groups of rats, the reversible effects upon withdrawal of NaF treatment and by administering some therapeutic agents, viz., ascorbic acid and calcium alone and in combination with NaF (50 and 70 days), on sperm structure and metabolism were also studied. The results revealed that the sperm acrosomal hyaluronidase and acrosin were reduced after 50 days of NaF treatment. Sperm stained with acidic alcoholic silver nitrate revealed acrosomal damage and deflagellation, which might be causative factors for the reduced activity of the enzymes. These alterations also resulted in a decline in sperm motility. The cauda epididymal sperm count was decreased, perhaps because of spermatogenic arrest. Thus, the low sperm motility and count ultimately contributed toward reduction in fertility by NaF treatment. However, withdrawal of NaF treatment for 70 days produced incomplete recovery, while administration of ascorbic acid and calcium, individually and in combination, brought about significant recovery of fluoride-induced effects. Thus, the effects of fluoride on sperm structure and metabolism of rats are transient and reversible. There were lack of differentiation and maturation of spermatocytes with marked infiltration in the interstitial area of seminiferous tubules and few mature spermatozoa were seen in the lumens of the seminiferous tubules in the NaF-treated group after 4 weeks and 6 weeks as compared to normal spermatogenesis showing various stages of differentiation and maturation with spermatozoa attached in groups to the inner aspect of the lumen of the seminiferous tubules in the control groups. These results were similar to earlier studies by Chinoy, *et al.* [25] which showed that 30 days of treatment with sodium fluoride (10 mg kg body weight) to mice resulted in sloughing off of the spermatogenic cells in the luminal regions of seminiferous tubules of the testis leading to disorganization of their epithelium that caused a complete absence of spermatogenesis in the testis. However, Sprando, *et al.* [1] reported that rats fed on 250 ppm fluoride for 10 weeks showed no distinguishable change in testicular histology from their control group. Shashi [27] evaluated relationship between infertility and the histological structure of the testes following the subcutaneous administration of different doses of sodium fluoride (5, 10, 20 and 50 mg/kg/day), for 100 days, to groups of six male albino rabbits; the six control animals were given 1 cc distilled water/kg b.w./day for the same length of time. Deficient maturation and differentiation of the spermatocytes and an increase in the amount of interstitial tissue were found in the experimental animals. In the higher dosage groups, spermatogenesis stopped and the seminiferous tubules became necrotic. The study thus established the existence of a definite relationship between fluorosis and testicular damage. Epidemiological investigations indicated that fluoride may cause adverse effects in the reproductive system of males living in fluorosis endemic areas, laboratory studies of Collins, *et al.* [28] found that fluoride does not adversely affect spermatogenesis or endocrine function at 25, 100, 175 and 250 mg/L in the drinking water of male rats.

Histopathological findings in this work were also in agreement with that of Wan, *et al.* [29]. They found that sperm density declined markedly at day 80 and day 120 of fluoride administration in the drinking water. Moreover, the number of seminiferous epithelial cell layers, the thickness and the diameter of the seminiferous tubule in the testes also decreased at day 50, 100, and 120 of the study. They summarized that the histological changes in the seminiferous epithelium of testicular tissues may be responsible for the diminished sperm quality in male rats. After both 4 weeks and 6 weeks, histopathology of testis in this present study supported the improvement in the spermatogenic activity in the NaF+Vit E-treated groups, NaF+Se-treated groups and NaF+Ge-treated groups but not much improvement in the NaF+Zn-treated groups compared to the NaF-treated groups. The extent of recovery was more pronounced with the groups fed on NaF+ Vitamin E where recovery was seen almost as that of the control groups.

This study revealed a significant decrease ($p<0.05$) in the testosterone level of the NaF-treated group (1.60 ± 0.17) after 4 weeks when compared to the control group (2.73 ± 0.11) but there was a significant increase in the NaF+Vit E-treated group (5.03 ± 0.15), NaF+Se-treated group (4.73 ± 0.25) and NaF+Ge-treated group (2.56 ± 0.15) but no significant difference in the NaF+Zn-treated group (1.76 ± 0.05) compared to the NaF-treated group (1.60 ± 0.17). After 6 weeks, the NaF-treated group (1.86 ± 0.05) showed no significant difference ($p>0.05$) in the testosterone level when compared to the control group (2.76 ± 0.25). Significant increase ($p<0.05$) was seen in the NaF+Vit E-treated group (6.76 ± 0.66), NaF+Se-treated group (6.90 ± 3.40) and NaF+Zn-treated group (6.53 ± 1.32) but no significant difference in the NaF+Ge-treated group (2.70 ± 0.75) when compared to the NaF-treated group (1.86 ± 0.05).

Prolactin level after 4 weeks showed a significant decrease ($p<0.05$) in the NaF-treated group (0.65 ± 0.06) compared to the control group ( 2.30 ± 0.36) but a significant increase in the NaF+Vit E-treated group (2.39 ± 0.18), NaF+Se-treated group (1.39 ± 0.01) and NaF+Ge-treated group (1.40 ± 0.18) but no significant difference in the NaF+Zn-treated group (0.70 ± 0.00) when compared to the NaF-treated group (0.65 ± 0.06). Significant decrease ($p<0.05$) was also seen after 6 weeks in the NaF-treated group (0.42 ± 0.01) compared to the control group (2.69 ± 0.07) but a significant increase in the NaF+Vit E-treated group (2.64 ± 0.16), NaF+Se-treated group (1.64 ± 0.10), NaF+Ge-treated group (1.38 ± 0.02 ) and NaF+Zn-treated group (0.82 ± 0.04) compared to the NaF-treated group (0.42 ± 0.01). Most significant amelioration was observed in the NaF+Vit E-treated group compared to other treated groups.

Luteinizing hormone level after 4 weeks revealed a significant decrease ($p<0.05$) in the NaF-treated group (0.27 ± 0.06) when compared to the control group(0.87 ± 0.07) but a significant increase in the NaF+Vit E-treated group (1.01 ± 0.16), NaF+Se-treated group (0.61 ± 0.09) and NaF+Ge-treated group (0.62 ± 0.02) but no significant difference in the NaF+Zn-treated group (0.33± 0.03) compared to the NaF-treated group (0.27 ± 0.06). After 6 weeks, a significant decrease ($p<0.05$) was still seen in the luteinizing hormone level of the NaF-treated group (0.34 ± 0.08) when compared to the control group (1.80 ± 0.03) but a significant increase in the NaF+Vit E-treated group (1.12 ± 0.10), NaF+Se-treated group (0.63 ± 0.08) and NaF+Ge-treated group(0.96 ± 0.12) but no significant difference in the NaF+Zn-treated group (0.42 ± 0.04) compared to the NaF-treated group (0.34 ± 0.08). Most significant amelioration was observed in the NaF+Vit E-treated group compared to other treated groups.

After 4 weeks, the follicle-stimulating hormone level showed a significant decrease ($p<0.05$) in the NaF-treated group (0.30 ± 0.02) when compared to the control group (0.76 ± 0.05) but a significant increase in the NaF+Vit E-treated group (0.88 ± 0.02), NaF+Se-treated group (0.77 ± 0.02) and NaF+Ge-treated group (0.79 ± 0.01) but no significant difference in the NaF+Zn-treated group (0.38± 0.05) compared to NaF-treated group (0.30 ± 0.02). After 6 weeks, there was still a significant decrease ($p<0.05$) in the follicle-stimulating hormone level of the NaF-treated group (1.01 ± 0.24) when compared to the control group (2.53 ± 0.05) but a significant increase in the NaF+Vit E-treated group (2.16 ± 0.18), NaF+Se-treated group (1.83 ± 0.06) and NaF+Ge-treated group (1.79 ± 0.01) but no significant difference in the NaF+Zn-treated group (0.93 ± 0.10) compared to the NaF-treated group (1.01 ± 0.2). Most significant amelioration was observed in the NaF+Vit E-treated group compared to other treated groups. Oritz-Perez's (2003), carried out a study where a group of individuals were exposed to fluoride concentrations at lower doses: 2-13 mg/day (low-fluoride-exposed group-LFEG) and 3-27 mg/day (High-fluoride-exposed group-HFEG). They observed that there was a significant increase in FSH ($p<0.05$) and a reduction of inhibin-B, free testosterone, and prolactin in serum ($p<0.05$) in the HFEG. When HFEG was compared to LFEG, a decreased sensitivity was found in the FSH response to inhibin-B ($p<0.05$). A significant negative partial correlation was observed between urinary fluoride and serum levels of inhibin-B ($r=-0.333$, $p=0.028$) in LFEG. Furthermore, a significant partial correlation was observed between a chronic exposure index for fluoride and the serum concentrations of inhibin-B ($r=-0.163$, $p=0.037$) in HFEG. No abnormalities were found in the semen parameters studied in his work, neither in the HFEG, nor in the LFEG. The results obtained indicated that a fluoride exposure of 3-27 mg/day induced a subclinical reproductive effect that can be explained by a fluoride-induced toxic effect in both Sertoli cells and gonadotrophs. A work by Susheela and Jethanandani [30] which focused on serum testosterone concentrations in patients with skeletal fluorosis, in order to assess the hormonal status in fluoride toxicity found out that circulating serum testosterones in skeletal fluorosis patients were significantly lower than those of Control 1 at $p < 0.01$. Testosterone concentrations of Control 2 were also lower than those of Control 1 at $p <0.05$ but were higher than those of the patient group. They concluded therefore that decreased testosterone concentrations in skeletal fluorosis patients and in males drinking the same water as the patients but with no clinical manifestations of the disease compared with those of normal, healthy males living in areas nonendemic for fluorosis suggested that fluoride toxicity may cause adverse effects in the reproductive system of males living in fluorosis endemic areas. In 1994, Narayana and Chinoy found out that the determination of circulating androgen levels in NaF-treated rats showed a downward trend compared to those of the control group, suggesting alteration in testosterone concentration. The histomorphometric studies revealed significant change in the Leydig cell diameter in correlation with the androgen levels. The results indicated that fluoride does interfere with steroidogenesis in short-term low dose exposures in rats. A marked fall ($p <0.01$) in the testosterone production was recorded at a fluoride concentration of 100 ppm and testosterone synthesis was maximally inhibited ($p <0.01$) at 200ppm. There was a noticeable, though marginal, inhibition in testosterone synthesis even at 10ppm fluoride concentration. From 1 ppm to 200 ppm, the degree of inhibition of testosterone synthesis seemed to be dependent on fluoride concentration as investigated by Kanwar, *et al.* [31]. Chinoy, *et al.* [32] revealed that the occurrence of giant cells in the lumen of mice testis after 30 days of treatment with fluoride and arsenic has been reported for the first time elsewhere [33]. In their study also, with fluoride+aluminium combined treatment, similar giant cells were observed denuded off from the spermatogenic epithelium into the tubular lumen. These giant cells could be the result of faulty or failed chromosomal replication or cell division. This again, to the best of their knowledge, is the first report of that effect. The treated animals also manifested decreased protein levels, probably correlated with structural changes contributing to faulty spermatogenesis in agreement with earlier data [33-35]. The significantly reduced activities of 3β- and 17β-HSDs in mice testis after fluoride+aluminium treatment for 30 days correlated with the significant accumulation of cholesterol. This, in turn, would reduce circulating testosterone levels [33] and alter the metabolism of all androgen-dependent reproductive organs in the animals studied [36]. Withdra wal of treatment for 30

days resulted in significant recovery in all the parameters investigated including histology, this corroborated our present study. However, in Group IV mice treated simultaneously with NaF+$AlCl_3$+vitamin C, complete amelioration of the induced toxic effects was observed, evidently due to the antioxidant and reducing properties of the vitamin which is known to increase cAMP levels promoting cellular growth and metabolism [33]. Their study therefore confirmed the beneficial effects of dietary factors in mitigating fluoride+aluminium toxicity in testis of mice. Susheela [37] also stressed the importance of antioxidants in the diet for recovery from fluoride toxicity.

These studies have shown that fluoride administration induces toxicity in the reproductive function of the test animals causing various degenerative and impaired changes in the sperm quality parameters of the test animals. It also revealed histological structural changes of the testis, including extensive lack of differentiation and maturation of spermatocytes and marked infiltration in the interstitial area of seminiferous tubules. Changes were also observed on the various reproductive hormones assayed for, which further indicated impairment of normal reproductive function in the albino rats. In the treated groups by some selected antioxidants, partial and complete amelioration of the induced toxic effects were observed, evidently due to the antioxidant and reducing properties of these antioxidants promoting cellular growth and metabolism. From this study, it is established that fluoride toxicity produced definite alteration on the testis, parameters of sperm and reproductive hormones which were duration dependent. Administration of selenium, ginseng and vitamin E supplements revealed significant recovery whereas treatment with zinc showed less improvement in the parameters which suggested that effects induced by NaF treatment were transient and reversible and hence no permanent damage occurred within the period of investigation.

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